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# Achievements and Challenges of Rrsearch on Truffles in China

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Abstract: Research on truffles has made a good progress in China since the last two decades. Before 1989 only 8 *Tuber* species were recorded. Since then, 25 species in *Tuber* and 1 in *Paradoxa* have been identified in China, of which are more than 50% new to science. More than 50 desert truffle species have also been recorded. These results suggest that China has a rich truffle flora and might be one of centres of truffle diversification and distribution. Chinese black truffles, the *T. indicum* complex, *T. aestivum* and *T. pseudoexcavatum* have been collected for consumption and trade locally for many years. But they were not scientifically described in China until recently. While Chinese black truffles they were virtually unknown internationally until the early 1990s when exports to international markets commenced. This has aroused great concern and interest in the study of Chinese black truffles. Research results indicated that *T. indicum* is the predominate truffle species exported to the international markets and a small amount of *T. pseudoexcavatum* mixed with them. Genetic and morphological variations of Chinese black truffle species are huge. *T. indicum*, *T. sinense* and *T. himalayense* are too closely related to each other that they are indistinguishable morphologically. The three species might be better called "the *T. indicum* complex". Harvesting and trading in Chinese black truffles is a multi-million dollar industry that has created considerable income for small rural communities and farmers. Unfortunately, natural forests and plantations in which truffles grow naturally have been damaged so much that created a big concern about their resources in danger. Protection of the precious resources is urgent! The first truffle plantation was established in Taiwan in 1989 and produced truffles in 1996. Other truffle plantations have been established in Hunan, Guizhou, Sichuan and Yunnan Province recently. It is said the first group of truffle ascocarps were produced at the Guizhou plantation in 2008, but the other plantations have not produced truffles yet. The production of truffle-infected trees and establishment of truffle plantations are underway and have considerable potential in China.

Key words: China; Truffles; Research; Cultivation

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## Diversity of Chinese truffle species

Research on truffles in last 20 years reveals that China has big diversity of truffles. However, Chinese ancient books had no records on truffle (*Tuber*) species. The first Chinese truffle species, *Tuber taiyuanense* to be recorded in China was in 1985 which was described by Prof. B. Liu. Since then, research on truffle diversity has expanded rapidly. Until now, 27 species and 1 variety have been recorded in China, of which 26 species and 1 variety in *Tuber* and 1 in Paradoxa. Among them the 23 *Tuber* species and 1 variety are "non-black coloured" and 3 are "Black coloured" truffle species (known as "Chinese back truffles"), 12 of those species are new to science, and 7 are similar but not identical to known species and so could be new species or subspecies (Wang, 1990; Zhang, 1990; Ren, 2003; Song, 2005; Wang *et al.*, 2006a; Chen, 2007; Chen and Liu, 2007; Chen *et al.*, 2008; Wang *et al.*, 2008). A new combination of *Parodoxa gigantospora* (Y. Wang & Z.P. Li) Wang was published in 2008 based on *Tuber* species, *Tuber gigantosporum* Y. Wang & Z.P. Li (1991) because

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its asci invariably contain only 1 spore . The first time of the genus of *Paradoxa* has been found in China and only the 2nd time that the genus has been found since 1935 when it was described by Mattirolo in Italy .

1 .“ Non-black coloured ”:

*Tuber asa* ?
*Tuber borchii* ?
*Tuber borchii* var. *sphaerospermum*
*Tuber californicum*
*Tuber chenggonense* #
*Tuber dryophilum* ?
*Tuber aestivum*
*Tuber excavatum*
*Tuber furfuraceum* *
*Tuber huidongense* *
*Tuber leptoperidium* #
*Tuber liaotongense* *
*Tuber liui* *
*Tuber lyonii* = *T. texense*
*Tuber maculatum* ?
*Tuber latisporum* *
*Tuber pseudorufum* #
*Tuber puberulum* ?
*Tuber rapaeodorum* ?
*Tuber rufum* ?
*Tuber taiyuanense* *
*Tuber umbilicatum* *
*Tuber zhongdianense* *

2 .“ Black coloured ”

The *Tuber indicum* complex:

*Tuber indicum*
*Tuber sinense*
*Tuber himalayensis*
*Tuber pseudohimalayense*
= *Tuber seudoexcavatum*
*Tuber formosanum* (nom . invalid)

3 . Parodoxa gigantospora

* New species, ? nov . sp . or subsp ., # not published sp . nov .

Chinese black truffles which are locally called as “ wu-niang-tong ” (fruiting body without mother plant) and “ song-mao-fuling ” (pine needle ’ fungus) have been collected for consumption and trade for many years in Sichuan and Yunnan (Zhang and Wang, 1990) . However, they were not scientifically described in China until 1989 when they were named as new species of *Tuber sinense* by Tao *et al* . In fact, *Tuber sinense* is almost identical to *T. indicum* which was described in Asian (Cooke and Massee, 1892) based on a truffle collection found near Mossorie in the northwest Himalayas of India . Ninety seven years later Zhang & Minter named another new species, *T. himalayense* based on a part of the same collection . In 1989 small amount of Chinese black truffles was exported to Germany for appraisal . Since then, increasing quantities were exported to international markets (Yamanaka *et al.*, 2001; Wang *et al.*, 2008), which has created concern and also interest in the study of these Chinese Tuber species (Fourné pers . comm, 1995; Wang and Hall, 2001) . Finally, it has been found that *T. indicum* is the predominate truffle species exported to the international markets and a small amount of *T. pseudoexcavatum* (Wang *et al.*, 1998) mixed with them . Last 16 years studies have revealed that genetic and morphological variations of Chinese black truffle species are huge . *T. indicum*, *T. sinense* and *T. himalayense* are too closely related to each other that they are indistinguishable morphologically (Paolociioni *et al.*, 1997; Mabru *et al.*, 2001; Song, 1995; Zhang *et al.*, 2005; Wang *et al.*, 2006a; Chen, 2007) . Therefore, it would be better to call them as “ the *T. indicum* complex ” (Wang *et al.*, 2006a, b; Wang *et al.*, 2008) . *T. pseudohimalayense* is a co-species of *T. pseudoexcavatum* (Chen, 2007) . *T. formosanum* (nom . invalid) was described as a new species by Hu (1992) based on a collection from Taiwan but is, in fact, not a valid species . Small amount of *T. aestivum* ( = *T. uncinatum*) is produced and sold with *T. indicum* locally in Sichuan Province (Fig.1: a, b) . It is only recently that *T. aestivum* ( = *T. uncinatum*) was distinguished from *T. indicum*, described scientifically and confirmed to be identical to the European collections by morphological and molecular methods (Song, 1995; Chen *et al.*, 1995) .

Additionally, over 50 non-tubers truffle species belonging to 28 genera, 15 families have been found in China in the last 20 years (Tao, 1988; Wang & Liu unpublished data) .

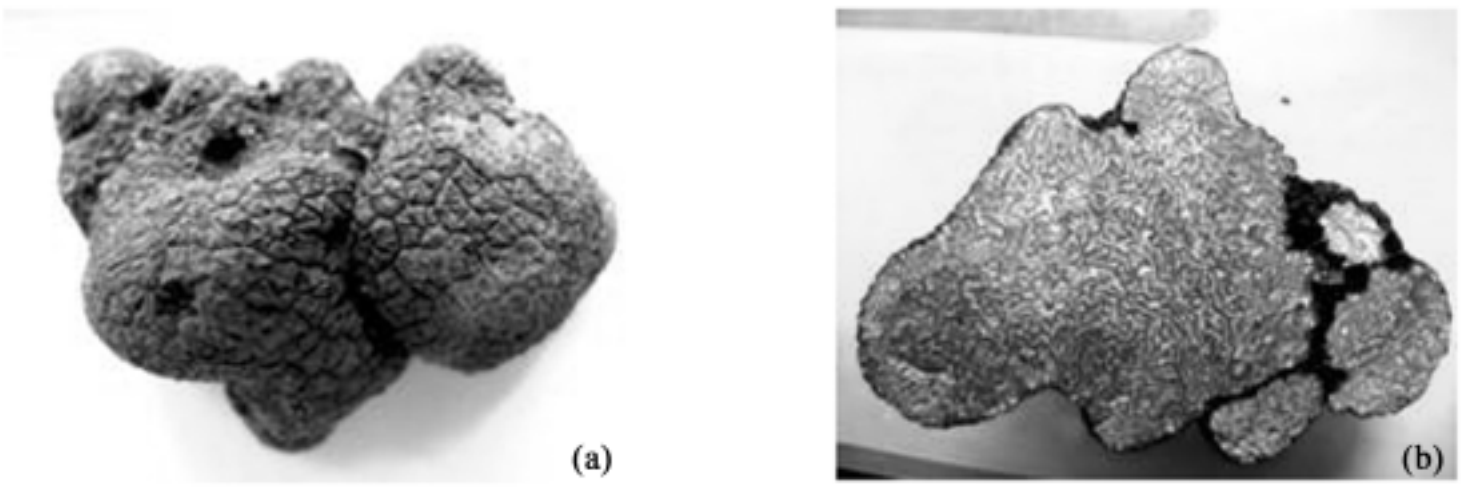


Fig.1 Ascocarps (a) and vertical section (b) of *Tuber aestivum*

ZYGOMYCETES
- *Endogne*

ASCOMYCETES
- *Elaphomyces*
- *Balsamia*
- *Choiromyces*
- *Fischerula*
- *Genebea*
- *Genea*
- *Geopora*
- *Hydnobolites*
- *Hydnotrya*
- *Pachphloeus*
- *Leucangium* (*Picoa*)
- *Terfezia*

BASIDIOMYCETES
- *Alpova*
- *Gautieria*
- *Gelopellis*
- *Gymnomyces*
- *Hydnangium*
- *Hymenogaster*
- *Hysterangium*
- *Melanogaster*
- *Octavianiana*
- *Phlebogaster*
- *Rhizopogon*
- *Richoniella*
- *Sclerogaster*
- *Zelleromyces*

Apparently, more research on non-tubers truffle species will be needed in China (Fig.2: a, b)

## Distribution of Chinese truffles

Only some part of Yunnan and Sichuan province and small areas in the other parts of China have been searched for truffles and so truffle distribution in China has been found to be predominately in south-west China and very spotty in the rest of China (Song, 2005; Chen, 2007; Chen and Liu, 2007; Wang *et al.*, 2008). The *Tuber indicum* complex, *T. pseudoexcavatum*, *T. aestivum*, *T. excavatum*, *T. huidongense*, *T. zhongdianese*, *T. borchii* var. *sphaerospermum*, *Tuber liui*, *Tuber umbilicatum*, *Tuber latisporum* and *Parodoxa gigantospora* grow in south-west China (Yunnan and Sichuan) and Taiwan. The rest of truffles have been found in both south (Yunnan, Sichuan, Tibet, Hubei and Taiwan) and north China (Jilin, Liaoning, Inner Mongolia, Beijing, Hebei, Shanxi, Gansu and Xinjiang). It is not surprised more truffle species and new distribution region will be discovered in China in the future.

## Natural truffle habitats

In northern China truffle species have been found in natural temperate forests and plantations, such as in coniferous forests or plantations of *Pinus*, *Larix*, *Abies* and *Picea* and also in deciduous broad-leaved forests of

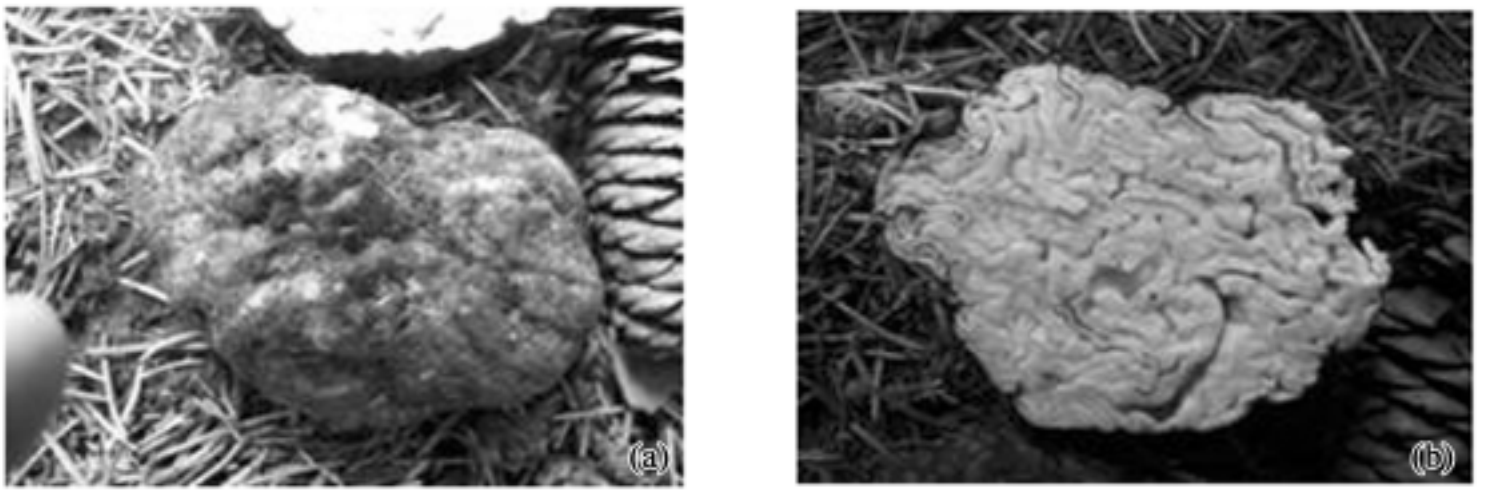


Fig.2 A fruit-body (a) and a vertical section (b) of the species *Geospora cooperi*

*Quercus*, *Carpinus*, *Tilia* and *Betula*, as well as in mixed forests of pine and deciduous broad-leaved trees. For example, truffles have been found in mixed forests of *Pinus koraiensis* with oak trees, plantations of *P. koraiensis* and *Larix gmelinii* in north east China; mixed forests of *P. tabulaeformis* with *Quercus mongolica*, *Q. mongolica* var. *liangtungensis* etc. and forests and plantations of *Picea* spp. in north China; forests and plantations of *Picea schrenkiana* and *Picea crassifolia* in north west China (Fig.3). Soils are usually acid.

In southern China, truffle species are found in subtropical evergreen broad-leaved forests of *Lithocarpus*, *Castanopsis* and *Quercus*, their second growth of coniferous forests and plantations of *Pinus*, *Keteleeria* and *Tsuga*, or in deciduous broad-leaved forests of *Quercus*, *Castanea*, *Carpinus* etc, and in mixed forests of pine and broad-leaved trees. Soils are usually calcareous or sometimes acid soils. Rainfall ranges from 300 to 600 mm annually.

The *T. indicum* complex, *T. aestivum* and *T. pseudoexcavatum* share the same habitats. They produced predominately from coniferous forests of *Pinus yunnanensis*, *P. armadii* and *Keteleeria evelyniana*, and their plantations (20 - 40 years old). The coniferous forests are the second growth of evergreen broad-leaved forests of *Castanopsis*, *Lithocarpus*, *Cyclobalanopsis* and *Quercus* (1600 - 2600 m). Chinese black truffles also grow in evergreen broad-leaved forests but production is much less. (Fig.4). *Coriaria nepalensis*, *Pyracantha fortuneana*, *Phyllanthus emblica* and *Ficus tikoua* are plants commonly found in Chinese black truffles producing areas which may be the indicators of the habitats. Alnus ferdinandi-coburgii is often found in the Chinese black truffles habitats under which production of ascocarps is higher than without them due to the soils with more moisten and N content. Soils are normally calcareous with a pH of 7.2 (5.3 - 7.9) and have a high content of Ca, Mg and Fe, and high ratio of Carbon to N (Chen, 2007). Compared with the soils where *T. melanosporum* inhabits the calcareous soils in south west China have lower pH due to heavily leaching and coniferous needles which bring organic acids to the soils. Chinese black truffles also inhabit in moderately acid to acid red soils which developed from lime stones or purple soli developed from purple sandy stones (Tang *et al.*, 2005; Wang unpublished data). Tang *et al.* (2005) did intensive investigation on habitats of Chinese black truffles in *Pazhihua*, Sichuan and discovered that Chinese black truffles preferred slightly acid oils with pH 6.0 - 6.5 on moderate slopes (20 - 30°) facing south. Though Chinese black truffles can inhabit in the acid soils but we believe they grow better and more productive in calcareous soils. García-Montero *et al.* (2008) and Geng *et al.* (2009) showed that *T. indicum* and *T. pseudoexcavatum* mycorrhizae grow well in calcareous substrates rich in active carbonate. Annual rainfall ranges from 600 to 1000 mm with more than 2200 annual sunny hours.

Chinese black truffles produce distinctive br lés as *T. melanosporum* does, inside which grass, herbaceous plants and small shrubs died (Fig.5). Ascocarps form at depth of soils of 0 - 30 cm.

## Chinese truffles and small mammals

Truffles produce fruit-bodies below ground and rely on animals, especially rodent, for dispersal of their spores (Johnson, 1996). When small mammals eat these truffles and deposit their spores elsewhere in their feces. Therefore, mammals play an important role in the maintenance of truffle mycorrhizal symbiosis and abundance in truffle forest ecosystems. Some small mammals opportunistically consume truffles and others consume truffles with high frequency. For example, northern flying squirrels (*Glaucomys sabrinus*)' fecal samples contains 90% over truffles spores (Colgan, 1997; Maser *et al.*, 1978). There are few small mammals have been seen when we looked for truffles in Yunnan forests, such as chipmunks, squirrels, flying squirrels, rabbits and mice (Fig.6). But there has been no any research on this important issue in China which is needed for better understanding and managing the truffle forest ecosystems.

## Mycorrhizas

Compared with *T. melanosporum* the *T. indicum* complexes have much bigger range of host plants of

Fig.3 Forests of *Picea schrenkiana* in Xinjiang region where *Geospora cooperi* was found

Fig.4 Natural Chinese black truffles forests of *Keteleeria evelyniana*

Fig.5 Brûle produced by *Tuber indicum*. Surrounding plants are *Eupatorium odoratum*

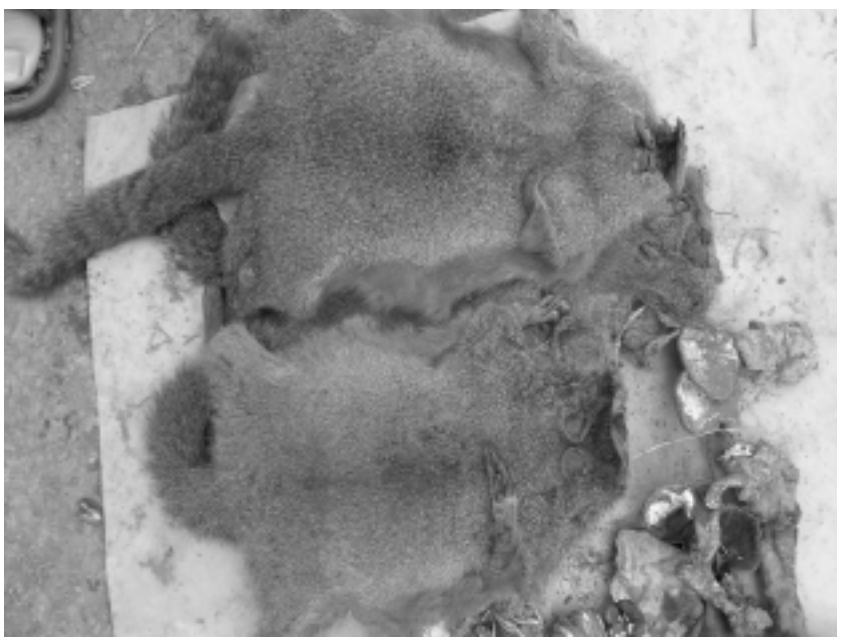

Fig.6 Flying squirrel skin on local market, Chuxiong, Yunnan

both broad-leaved and coniferous trees. However, only a few ectomycorrhizal associations have been studied and confirmed by morphological and molecular methods, such as with *Pinus yunnanensis*, *P. armadii*, *Keteleeria evelyniana*, *Quercus franchetii* and *Castanea mollissima* (Deng, 2009). The ectomycorrhizas formed by the *T. indicum* complex are morphologically very similar to the ones produced by *Tuber melanosporum* in their vertical branching emanating hyphae and puzzling-like mantle structure (Manjón *et al.*, 1998; García-Montero *et al.*, 2008; Geng *et al.*, 2009; Deng, 2009). The *T. indicum* complex were synthesized with Europenan tree species, such as *Pinus pinea*, *Quercus cerris*, *Quercus pubescens*, *Q. ilex* and *Quercus ilex* L. subsp. *ballota*. The mycorrhizas were very similar to the ones by *Tuber melanosporum* (Zambonelli *et al.*, 1996, 1997; Di Massimo *et al.*, 1998; Manjón *et al.*, 1998; García-Montero *et al.*, 2008).

## Kill the hen to get the eggs-truffle resources in danger

Hundreds tonnes of Chinese black truffles are produced annually in China now. Harvesting and trading Chinese black truffles are becoming a multi-million dollar industry (Fig.7) that has brought good income to the local mountainous economy and farmers (Wang *et al.*, 2008). Good amounts of Chinese black truffles have been exported into European, American and Japanese markets. Unfortunately, plundering commercially harvesting driven by greed has brought big damage to

the habitats . The forest floors looked like deeply ploughed farm soils, tree roots were cut off and exposed to the air after the soils were dug up for searching truffles 3 - 4 times a day (Fig. 8) . The damage resulted in sharply declining of the truffle production or no any production at the next few years or forever ! To change the destructive harvesting methods is quite urgent . Careful management of truffle industry to protect truffle resources in China is very necessary . To train truffle dogs for hunting truffles and issue regulations for management of truffle resources has just stated in China .

Research on production of truffle seedlings and establishment of truffle plantations Research on producing truffle-infected seedlings for establishing plantations has been recently carried out . The technology was either introduced from overseas or developed by Chinese research institutions (Hu *et al.*, 2006; Lin *et al.*, 2008; Geng *et al.*, 2009) . Seedlings of *Pinus yunnanensis*, *P. armandii*, *Castanea mollissima*, *Quercus franchetii*, *Q. aliena*, *Cyclobalanopsis glauca*, *Corylus yunnanensis* and *Castanopsis delavayi* haver been successfully mycorrhized with *T. indicum* under greenhouse conditions . *Q. aliena* and other Chinese tree species have also successfully been mycorrhized with *T. melanosporum* . Research on technology for inoculating other Chinese oak species with *T. melanosporum* and *T. aestivum* is underway .

The first truffle plantation was established on acid soils in Taiwan in 1989 . The soil was limed with quick lime (5.0 t ha in 1987 and 1.7 t ha in 1988 and 1989 respectively) to bring the soil pH up from 4.5 to 7.0 . Two-years-old seedlings of *Cyclobalanopsis glauca* infected by *Tuber formosanum* ( = *T. indicum*) were planted at a spacing of 6 × 6 m . Weeding was carried out three times a year . The truffle trees were pruned when they were four years old . No irrigation was applied . The first group of ascocarps was found in 1996 and 10 kg of truffles were harvested from 30 truffle trees in the following year . Br lés formed around the trees where the ascocarps were found . Contaminated ectomycorrhizas have developed since 2 000 and fruiting bodies of *Scleroderma areolatum* and *Rhizopogon* sp . have been found since .

The second truffle plantation was established in Cili County, Hunan, China, in 2002, with *T. melanosporum*-infected hazel (*Corylus avellana*) and chestnut trees (*Castanea mollissima*) at space of 3 × 3 m . The hazel trees were obtained from France and chestnut trees were produced from the Institute of Forest Ecology and Environment, Hunan Academy of Forestry, China . The soil was acid and limed to raise the pH from 4.5 to over 7.0 . Six more truffle plantations have been established since 2002 . One of them weas established with *Quercus aliena* infected by *T. indicum* and *T. melanosporum* at calcareous soils in Guiyang, Guizhou in 2004 . Two of them were established in *Panzhihua*, Sichuan in 2007 with *T. indicum* infected seedlings of *Pinus armandii*, *Pinus yunnanensis* and *Castanea mollissima*, on acid soils (Fig. 9) . Three of them were in Yunnan in 2008, one of which was in Lufeng County, Yunnan with two years old *T. indicum* infected seedlings of *Quercus franchetii* and *Castanopsis delavayi* on acid red soil . The trees were produced by the Chuxiong Forest Research Institute . The soil was limed with quick lime only around the trees . The other three plantations have been set up in the Kunming suburb and northwestern Guizhou and were planted with *T. indicum*-infected *Pinus armandii* and *Castanea mollissima* (at 5 × 5 m) which produced from Kunming Institute of Botany, Chinese Academy of Sciences since 2007 . The soils were acid red soil and limed with smash limestone (around trees, 80 × 80 cm) to raise the pH to around 7.5 . The six truffle plantations have not produced yet except the on in Guizhou which produced its first group of ascocarps of *T. indicum* and *T. melanosporum* in December 2008 (Hu pers . comm .) .

Cultivation of truffles in China is just unfolding . However, south-west China has large areas of lime-stone soils and suitable climate for truffle growth where cultivation of truffles has boundless prospects (Fig. 10) .

## Conclusions

(1) The results of research on truffles in the last 20 years revealed that China, in particular south-west China has a huge diversity in truffle populations, genetics

Fig.7 Sacs of Chinese black truffles at local truffles market, Miyi county, Panzhihua, Sichuan

Fig.8 Severe damaged truffle forest floor of *Pinus armandii*

Fig.9 *Tuber indicum* plantation established at Panzhihua region, Sichuan in 2007

Fig.10 Lime stone landscape, Kunming, Yunnan

and habitats which represents a key centre of truffle origin, differentiation and distribution .

(2) A good example of the diversity of Chinese truffles is the extremely big variations of morphology and genetics of *T. indicum* complex . Three or four names have been proposed for this complex . DNA sequencings consistently divide them into two clades (subgroups), but these two clades can not be distinguished morphologically (Wang *et al.*, 2006a, b; Song, 2005; Yang *et al.*, 2005; Cheng, 2007; Deng, 2009) . The diversity is driven by habitat and plants diversity . In China, in particular in south-western China, the Himalayas and Hengduan Mountains have formed since the Cenozoic Era, creating a comprehensive climatic conditions from tropical, subtropical, temperate to alpine cold-temperate (including elevation changing from 56 to 7 700 m) and a all the vegetative types from tropical rain forests to cold-temperate coniferous forests . These areas were somehow protected from the impact of the last ice age, creating an ideal refuge for living creatures, including truffles . These natural conditions have made this region as one of the key centres of origin, differentiation and distribution of tree species of Pinaceae, Fagaceae, Betulaceae, Corylaceae and Salicaceae (Lu, 1999) all which are important host plants of truffles and therefore, the region might be one of centres of origin, differentiation and distribution of truffles .

(3) Close biogeographic relationships between south-western China and the Mediterranean in truffle

flora. For example, the some of truffle taxa, such as *T. aestivum*, *T. excavatum*, *T. borchii* var. *spherosperma* have been found in both regions. Likewise, the Tuber indicum complex in south-western China, quite resembles the European Périgord black truffle (*T. melanosporum*) (Yamanaka *et al.*, 2001; Chen, 2007; Jeandroz *et al.*, 2008). *T. pseudoexcavatum* from south-western China is closely related to the European species *T. brumale* (Jeandroz *et al.*, 2008). *P. monospora* is known only from Italy and *P. gigantospora* only from south-western China (Wang *et al.*, 2008).

(4) Chinese black truffles (The *T. indicum* complex) resemble Périgorde black truffle (*T. melanosporum*) not only morphologically and genetically but also their mycorrhizas (Zambonelli *et al.*, 1996, 1997; Di Massimo *et al.*, 1998; Manjón *et al.*, 1998; García-Montero *et al.*, 2008; Geng *et al.*, 2009; Deng, 2009). They may have the same ancestors (Jeandoz *et al.*, 2008).

(5) Chinese black truffles are important resources for the local economy and farmers in China and also necessary complement to deficit international truffle market, in particular European truffle markets. Chinese black truffles are one of good edible truffles even though their aroma is not strong as *T. melanosporum* (Bellesia *et al.*, 2002). Compared with *T. melanosporum*, *T. indicum* has about the same nutritional value and even better in the vitamin, mineral and amino acid contents (Yang, 2001).

(6) To change the destructive harvesting methods and careful management of truffle industry to protect truffle resources in China are urgently needed.

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